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Toxicogenomic study in rat thymus of F1 generation offspring following maternal exposure to silver ion

Xiugong Gao*, Jeffrey J. Yourick, Vanessa D. Topping, Thomas Black, Nicholas Olejnik, Zachary Keltner, Robert L. Sprando

Division of Toxicology, Office of Applied Research and Safety Assessment, Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, Laurel, MD, USA

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ABSTRACT

Male and female rats (26-day-old) were exposed to 0.0, 0.4, 4 or 40 mg/kg body weight silver acetate (AgAc) in drinking water for 10 weeks prior to and during mating. Sperm-positive females remained within their dose groups and were exposed to silver acetate during gestation and lactation. At postnatal day 26, the effect of silver ions on the developing F1 generation rat thymus was evaluated at the transcriptional level using whole-genome microarrays. Gene expression profiling analyses identified a dozen differentially expressed genes (DEGs) in each dose group using a loose criterion of fold change (FC) >1.5 and unadjusted $p < 0.05$, regardless of whether the analysis was conducted within each gender group or with both gender groups combined. No dose-dependent effect was observed on the number of DEGs. In addition, none of these genes had a false discovery rate (FDR) <0.05 after correction for multiple testing. These results in combination with the observation that thymus-to-body-weight ratios were not affected and no histopathological abnormalities were identified indicate that *in utero* exposure to silver ions up to 26.0 mg/kg (equivalent to 40.0 mg/kg silver acetate) did not have an adverse effect on the developing thymus.

1. Introduction

Silver has been used for centuries as a biocidal material. In ancient time, silver was used to preserve water in the form of silver vessels or silver coins. Its medical use was documented as early as 750 AD. Starting from the seventeenth century, silver has been used as antiseptics in a number of medical situations such as cholera, eye infection, and burn wound [2]. The U.S. Food and Drug Administration (FDA) approved the use of charged silver solutions (*i.e.* electrocolloidals) as antibacterial agents in the 1920s. The application of silver was further expanded during the second half of the twentieth century as a disinfectant in conjunction with hydrogen peroxide.

Today, silver-containing products are used in a wide range of healthcare, food industry, and domiciliary applications and are commonly found in hard surface materials and textiles. In the food industry, silver-containing compounds or their mixtures are widely applied onto food-packaging materials, often in direct contact with the food. Such an extensive use of silver raises concerns about its safety, toxicity, and health risk. However, there is a paucity

Abbreviations: AGCC, Affymetrix GeneChip Command Console; AgAc, silver acetate; AgNP, silver nanoparticle; DEG, differentially expressed gene; FC, fold change; FDR, false discovery rate; HCA, hierarchical cluster analysis; NOEL, no observed effect level; NK, natural killer; PCA, principal components analysis; RMA, robust multi-array average; SV, source of variance.

* Corresponding author at: 8301 Muirkirk Road, Laurel, MD 20708, USA. Tel.: +1 301 796 0580.
E-mail address: xiugong.gao@fda.hhs.gov (X. Gao).

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of information on the toxicity of silver. It is well known that ingesting large amount of silver preparations, which rarely happens, results in argyria, manifested by an irreversible gray to blue-black coloring of the skin due to subdermal silver deposit [11]. Also, the use of ionic silver and silver derivatives for treatment and prevention of infection of burn wounds or skin grafting has been associated with a number of side effects such as cytotoxicity, staining, methaemoglobinaemia and electrolyte disturbance, longer slough separation time, retardation of wound healing, and the possible inactivation of enzyme debriding agents [4]. The free silver ion (Ag^+) is the most toxic species of silver. Toxicity testing in fathead minnow (*Pimephales promelas*) showed that free silver ion was about 15,000 and 300 times more acutely toxic than silver sulfide and silver chloride complexes, respectively, which are the major forms of silver in the environment [9]. However, the toxic effect of long-term exposure to low concentrations of silver has not been well studied.

There is a potential risk for the developing fetus when pregnant women are exposed to silver products. A case–control epidemiology study was conducted by [1] among women who delivered infants from 1977 to 1980 in a Massachusetts hospital. Trace element levels of public water were analyzed from the communities in which the women resided during pregnancy. The relationship between community drinking water quality and the occurrence of late adverse pregnancy outcomes was examined. After adjustment for confounding factors, the results suggested some association between maternal exposures to 0.001 mg/L of silver in the drinking water (1/100 of the EPA standard) and some increase in fetal developmental anomalies (ear, face, and neck). As the authors recognized, there are inferential limitations to epidemiologic studies and further research is needed to explore these findings.

The U.S. FDA evaluated data available on the use of silver mixtures as antimicrobial agents in food contact polymers and suspected that *in utero* exposure to silver may have an adverse effect on the immune system of the developing animal. A comprehensive study for risk assessment has been conducted in our group using a rat model and conventional toxicological and/or pathological endpoints to confirm that the previously observed adverse effects are due to silver ion alone, and to define the no observed effect level (NOEL) at or below which the adverse effect does not occur [16].

Microarray technology has become a powerful tool to explore the expression levels of thousands of genes or even complete genomes after exposure to toxicants and has thus found wide applications in toxicological research [15]. Toxicogenomics, defined as the "global analysis of gene expression in target cells or tissues in response to a toxicant," has emerged as a promising approach to evaluate mechanisms of action in toxicological models [5]. Information on the global gene expression profile may provide clues to understanding biological actions of toxic substances at the molecular, cellular, tissue, and individual animal levels. Tissue-specific gene expression profiles can provide a basis for understanding tissue function, enabling molecular characterization of differences between normal and diseased tissue. Toxicogenomics also provides opportunities for improvements in toxicity screening and risk assessment such as the development of new predictive models for identifying human health hazards and the identification of more precise molecular biomarkers of exposure [12]. In this application, toxicogenomic approaches usually are more sensitive than conventional toxicological endpoint assays and can assess toxic responses at low doses and at the very onset.

As a component of the comprehensive research [16], the current study used a toxicogenomic approach to study the effect of silver ions on the developing thymus at the transcriptional level by using whole-genome microarrays to study global gene expression changes in rat thymus of F1 generation offspring from dams exposed to different levels of silver ion. In the last years, some dozen reports appeared in the literature using toxicogenomics approach to study silver toxicity; however, the majority of these studies were on silver nanoparticles (AgNPs). Only a few evaluated silver ion toxicity either in a crustacean model [13], an *in vitro* fish model [20], or in bacteria [21]. Silver ions and AgNPs exerted toxicity through different mechanisms; the latter was affected by several other factors other than silver ion itself, including surface coating and particle size [13]. To our knowledge, this study represents the first evaluation of silver ion toxicity using toxicogenomics approach in a mammalian model.

2. Materials and methods

2.1. Experimental design

Thymus tissues were obtained from 5 male and 5 female F1 generation rat pups on postnatal day 26 in each treatment group that were randomly selected from the animals evaluated in [16]. In brief, 28-day-old male and female CD IGS VAF/+ rats were exposed *ad libidum* to Hydro-System water containing 0 (control), 0.4, 4.0, or 40.0 mg/kg body weight silver acetate for 10 weeks prior to mating and during the 2–3 week mating period. Sperm-positive females remained within their dose groups and were further exposed to silver acetate throughout the gestation and lactation periods. Pups were weaned on lactation day 21 and euthanized for tissue collection on day 26. To minimize potential litter effect, no more than one pup was randomly selected from each litter within a treatment group.

2.2. Tissue collection, RNA isolation, and quality assurance

Thymus from each pup was collected and snap-frozen in liquid N_2 with 5 min after dissection. The samples were kept in a −80 °C freezer until processing for total RNA extraction. Thymus was disrupted using the TissueLyser (Qiagen, Valencia, CA) in the QIAzol Lysis Reagent (Qiagen) and total RNA was isolated on the EZ1 Advanced XL (Qiagen) automated RNA purification instrument using the EZ1 RNA Universal Tissue Kit (Qiagen) following the manufacturer's protocol, including an on-column DNase digestion. RNA concentration and purity (260/280 ratio) were measured with the NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). Integrity of RNA samples was assessed by the Agilent 2100 Bioanalyzer